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Oxytocin and Social Preference in Female House Mice (*Mus musculus domesticus*)

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Abstract

In social species, same-sex individuals may form social bonds behaviourally expressed as individual preferences, resulting in fitness benefits such as increased offspring survival, longevity and group cohesion. As a result of individual preferences, female house mice (*Mus musculus domesticus*) form social affiliations while communally nursing and may do so with kin or non-kin. However, the mechanisms behind the formation of such preferences are unknown. Oxytocin has been linked to a range of social behaviours including bond facilitation, social memory and parental care. Here, we experimentally increased oxytocin in pairs of unfamiliar, unrelated females and predicted that females with elevated oxytocin would demonstrate increased affiliative behaviours compared against a control. Subsequently, we tested for the formation of a social preference, using a preference test with the previous partner and a new unfamiliar female. Our results indicated no significant effect of treatment on positive and negative behaviours between females during the three initial cohabitation days. In both treatments, females demonstrated increased socio-positive behaviours and cohabitation time with their partner and decreased socio-negative behaviours and latency to meet, over the 3-d period. During the partner preference test, control but not oxytocin females demonstrated a significant preference for their cohabitation partner, and oxytocin females spent similar amounts of time with both stimulus females. Therefore, increasing peripheral oxytocin appears not to be involved in the facilitation of initial encounters with a stranger but may hinder the formation of a preference for this new partner.

Introduction

Same-sex individuals that establish bonds with their conspecifics can benefit from increased offspring survival, longevity, decreased stress and increased group cohesion (König 1994; Silk et al. 2003; Yee et al. 2008; Seyfarth & Cheney 2012). Typically, time spent in close association has been considered as an indicator of social bonding between individuals, and benefits have been documented in a wide variety of species including female savannah baboons (*Papio cynocephalus*) who gain greater offspring survivorship when individuals are more socially integrated (Silk et al. 2003), and female house mice (*M. musculus domesticus*) who when nursing with a preferred female partner have greater reproductive success (Weidt et al. 2008). Positive correlates between sociality and reproductive success have also been found in female horses, *Equus equus* (Cameron et al. 2009), bottlenose dolphins, *Tursiops truncatus* (Frère et al. 2010), and male macaques, *Macaca assamensis* (Schülke et al. 2010). These findings, among others, have demonstrated and brought to light the benefits associated with sociality and bonding with same-sex conspecifics. Therefore, to benefit from such fitness advantages, social partnerships are believed to be important.

The mechanisms behind choosing a social partner and establishing social partnerships are poorly understood; however, a number of processes may be

involved. One such mechanism is the use of physical and physiological cues in which a conspecific's relatedness, breeding status or ability to produce offspring (Hurst 1990; Weidt et al. 2008) could be determined. Relationships between non-kin have been linked to endocrinological mechanisms, in particular that of the oxytocinergic system (Beery & Zucker 2010; Wittig et al. 2014). In humans and other mammals, the peptide hormone oxytocin has been linked to a range of social behaviours including the facilitation of bonds between a mother and her offspring and between mating partners (reviewed in: Anacker & Beery 2013). In particular, oxytocin has been extensively studied for its effects on pair bonding in the monogamous prairie vole, where it was demonstrated to play a role in facilitating pre-copulatory bonding between males and females and increased social contact (Williams et al. 1994; Insel & Hulihan 1995; Cho et al. 1999; Ross & Young 2009).

Increased interest in oxytocin over recent years has led to a variety of studies related to social behaviours. Manipulation of oxytocin levels by injection increased huddling behaviour of females towards an unfamiliar female in meadow voles, *Microtus pennsylvanicus* (Beery & Zucker 2010); increased a range of cooperative behaviours such as digging, guarding and pup feeding in meerkats, *Suricata suricatta* (Madden & Clutton-Brock 2011); and increased investigatory behaviour and time in close proximity with familiar conspecifics, in naked mole rats, *Heterocephalus glaber* (Mooney et al. 2014). Additionally, elevated levels of oxytocin were found in chimpanzee, *Pan troglodytes*, urine following grooming behaviour with preferred social partners (Crockford et al. 2013), and after food sharing with conspecifics (Wittig et al. 2014). These studies, among others, suggest that oxytocin aids in facilitating bonds between known individuals and acts to strengthen these relationships. Conversely, various studies in oxytocin knockout mice caused a lack of social memory (Ferguson et al. 2000, 2001) and increased aggression (Winslow & Insel 2002). Therefore, oxytocin is a prime candidate to study its role in choice of same-sex social partners.

House mice offer an ideal study species to investigate choice of social partner as they are included in a small percentage of mammals that rear their offspring via communal nursing, when two or more females cooperate and indiscriminately nurse their offspring in the same nest (König 1989; Packer et al. 1992; Hayes 2000). Research has shown that female mice preferentially nest with familiar sisters forming egalitarian relationships that increase lifetime reproductive success for both females (König 1994). Females also form preferences for individuals when kept in groups of unrelated females, where they will establish communal nests and have greater success when nursing with a preferred partner (Weidt et al. 2008). Therefore, it is believed that females choose social partners to communally nurse with and do so when the most suitable partner is available. Females in a free-living environment are also selective in their partner choice and when given the option only choose to nest communally in 33% of cases (Weidt et al. 2014). In semi-natural, outdoor enclosures, communal nursing occurs in up to 90% of cases (Manning et al. 1995). Time spent together before communally nursing is suggested to be the best indicator for partner preference (Weidt et al. 2008). However, regardless of whether or not females nest with kin or non-kin, the mechanisms involved in choosing a communal nursing partner are not yet known.

In this study, we experimentally increased oxytocin, over a period of 3 d, in pairs of unrelated, unfamiliar females and predicted that females with elevated oxytocin would demonstrate increased affiliative behaviours when compared against a control. Subsequently, we tested for the formation of a social preference using a preference test, with a choice between the previous partner and another unfamiliar female. We thus aimed to understand whether oxytocin could influence the initial behaviours females exhibit towards an unfamiliar female and determine whether it can facilitate establishment of a preference.

Methods

Subjects

We used 48 female house mice that were sexually mature but non-breeding (virgin). Animals were laboratory-born F1 to F3 descendants of wild house mice (*M. musculus domesticus*) from a barn population near Zurich, as described in König & Lindholm (2012). Animals were weaned at day 23 and kept in same-sex sibling groups where they remained until 8–10 wk of age; in rodents, the oxytocin system is fully developed at weaning (Yamamoto et al. 2004). Each cage contained standard animal bedding (Lignocel Hygienic Animal bedding, JRS), with *ad libitum* cardboard and tissue for bedding and shelter. Mice were kept at a temperature of 22–24°C and humidity of 50–55%, under a constant light–dark cycle of 14:10 h (lights on at 05:30 h CET, with a half hour dawn and dusk phase at the beginning and end of the light phase). At all stages, food (laboratory animal diet for mice; Provimi Kliba SA, Kaiseraugst, Switzerland) and water was

provided *ad libitum*. To avoid excessive manipulations, we did not check for oestrous cycles or ovariectomize the females. When wild-derived female mice are housed in the conditions described above, 70% of females do not show ovarian cycles (Weidt 2007). Given that females were randomly assigned to the different injection treatments, endogenous oestrogen levels should be low and overall similar between treatments. Animal use and experimental design were approved by the Veterinary Office Zürich, Switzerland (no. 34/2014; Kantonales Veterinäramt, Zürich).

Experimental Procedures

In total, the experiment lasted for 5 d, which comprised of a cohabitation phase lasting 3 d followed by a 7–8 h separation period and a 1-d preference test.

Phase A: Cohabitation

Pairs of unfamiliar genetically unrelated females were randomly assigned to one of two groups, oxytocin (OT) or saline control (CON); both females in a pair received the same treatment. Unfamiliar, unrelated females were chosen, as we wanted to understand what mechanisms promote initial preference formation; familiar or related females could have established a preference prior to the start of the experiment. Female pairs were matched, where possible, in age (age difference: 7 ± 10 d, mean ± SE) and weight (weight difference: 2.9 ± 1.3 g, mean ± SE). Injections were given on three consecutive days between 16:00 h and 18:00 h prior to the dark phase (lights out at 20:30 h, during dusk and dawn mice are most active, Mackintosh 1981). To keep stress to a minimum, all females were restrained in a secure, one-hand technique. Due to all females experiencing the same restraint, we believe any stress effects on behaviour should be similar for all females. Following each injection, females were allowed a 15-min recovery period in a Makrolon Type II cage (width: 18 cm, length: 24 cm, height: 14 cm; made of transparent polycarbonate plastic), and afterwards, the cages of the pair being observed were connected with a transparent plastic tube, allowing both females access to both cages. As soon as both cages were connected, behaviours were video recorded (Sony camcorder) for later analysis, with red light allowing video recordings during the dark phase (20:30 h to 06:30 h).

Using the video footage, a series of observations were carried out. Latency for the two females to meet was recorded once the cages were joined, and after the first interaction, a 60-min behavioural focal followed. In addition, 12 10-min behavioural focals were made at the beginning of every hour (19:00 h to 06:00 h). The longer initial focal observation was chosen based on the results obtained by Neumann et al. (2013), where a peripheral OT injection led to elevated circulating OT for the first 2 h post-administration. All occurrences of behaviours and their duration were recorded for the pair together. For analysis, these behaviours were grouped into four main categories: socio-positive, socio-negative, neutral behaviours and time in the same cage (see Table 1, for detailed behavioural descriptions). As negative interactions occurred quickly (lasting less than a minute), we chose to analyse counts of negative interactions and time of positive interactions (as duration of positive behaviours lasted for longer periods and provided a more accurate measure). Time spent in the same cage was also analysed, and scoring was completed blind to treatment group. Additionally, we recorded whether the females were in the same or different cage on every half hour throughout the night, and at three time points (09:00, 12:00 and 16:00 h) during day light hours.

Peptide and doses

Synthetic oxytocin (Product: O4375 - 250 IU; Sigma Aldrich Co., Germany) was dissolved in sterile saline

Table 1: Description of socio-positive, socio-negative and neutral behaviours recorded during cohabitation, behavioural observations

Behaviour	Description
Socio-positive	
Huddle/rest	Resting in side-by-side contact
Allogroom	Grooming, being groomed or both females groom each other
Side-by-side contact	Eating or climbing side by side, not resting or huddling
Investigatory	Sniffing other; nose, ano-genital or elsewhere (positive when not followed by chase or fight)
Follow	Following the other (walking), female being followed is not running
Socio-negative	
Chase	Pursuing the other (running) or aggressively running towards other
Flee	Rapidly moving away from other (running)
Fight	Bite or attacking other
Submissive	One female to the other, rearing on hind legs
Tail rattling	Rapid side-to-side movement of the tail
Neutral	
Rest separately	Both resting in the same cage as each other, no body contact
No interactions	Both in same cage but no interaction, for example one in cardboard shelter, other active

(0.9% NaCl; Bischel) to give a concentration of 0.12 mg (or 2 IU)/ml (approximately 0.6 mg/kg). Subjects on each of three testing days received an intraperitoneal (i.p.) injection of either OT or CON. Half of the animal pairs (n = 12) received OT (0.012 mg OT/0.1 ml saline) and the remaining pairs (n = 12) received an equivalent dose of isotonic saline (0.1 ml). Both animals in a pair received injections of the same treatment. Dosage of OT was derived from a previous study where i.p. administration of OT increased brain levels of OT in the 30 min following injection (Neumann et al. 2013).

Phase B: Partner preference

On the fourth day at 08:30 h, the morning after the third injection, each pair was separated. During this separation period, females were kept in their home cage and had olfactory information about their partner. This separation period was decided upon as it meant females had no physical contact with their partner for a couple of hours and would help indicate a clearer preference during the preference test. At 14:00 h, the focal female was placed into the central cage of the partner preference set-up to allow acclimatisation. At 16:00 h, this cage was connected with transparent tubes to the cages with the stimulus females, and the focal female was then able to access all three cages. In total, females were in the preference test for 18 h.

The partner preference set-up consisted of two Makrolon Type III cages (width: 23.5 cm, length: 39 cm, height: 15 cm) connected with transparent plastic tubes to a central Makrolon Type II cage (Fig. 1). Each of the Type III cages was bisected laterally with a wire mesh barrier. Stimulus females were placed in the contained halves of the Type III cages. The focal female was placed in the centre cage and had access to all three cages. She was able to interact with stimulus females through the mesh via the use of visual and olfactory cues. The stimulus females could not interact with each other. The focal female was randomly chosen from the treated pair. Stimulus females were the focal female's previous partner, and a new, unfamiliar, genetically unrelated female, matched in age (age difference: 11.0 ± 2.09 d, mean ± SE) and weight (weight difference: 2.6 ± 0.38 g, mean ± SE); position of stimulus females was randomly assigned.

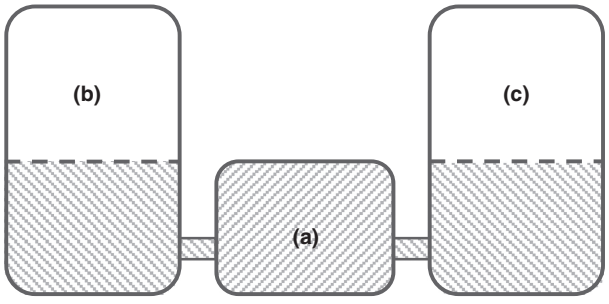

Fig. 1: Illustration of the partner preference set-up. (a) The area accessible to the focal mouse (grey-coloured sections). (b and c) Cages were bisected laterally with a mesh barrier confining them to the white zone. (b) The cohabitation partner of the focal and (c) represents a new unfamiliar, unrelated stimulus female; (b and c) were randomly assigned to the left or right side for each new preference test. (a) Could interact with both stimulus females via olfactory and visual cues.

Partner testing

The partner preference began once the focal female had placed at least her two front paws in both sides of the set-up (Fig. 1, grey section of (b) and (c)). The side she visited first and the latency to enter each side were recorded. Video recordings were made and a computer app (D.A.T.A, version 1.0.8; Behavior Science.org, LLC) allowed for the scoring of time the focal spent in each cage. Videos were scored blind to treatment group to avoid any bias in the results. Time spent in each cage was recorded for an hour after the test started and then for alternate hours throughout the preference test. Each observation hour began at half past the hour, totalling 9 h of observation per pair. As in previous studies, a social preference was defined as the focal female spending significantly more time with one female over the other (Carter et al. 1992; Williams et al. 1994; Insel & Hulihan 1995; Young & Wang 2004).

Two pairs from phase A were excluded from the partner preference test due to one of the animals breaking through the mesh barrier (both from the control group).

Statistical Analyses

Statistical tests were carried out using R version 3.1.3 (R Core Team 2015). To analyse time spent (seconds) interacting, either positive or in the same cage and for latencies to meet, we used linear mixed-effects models (hereafter: LMM). Occurrence of negative behaviours was analysed with a generalised linear mixed-effects model (hereafter: GLMM) using the MASS package in R (Venables & Ripley 2002), and negative binomial was used to correct for over dispersion (Ismail & Jemain 2007). Correcting the occurrence of negative

behaviours for time females spent in the same cage yielded the same result as the raw number of behaviours, and we therefore used the latter. The proportion of time females spent in the same cage at different time points throughout the day was analysed using a GLMM with binomial error distribution. For all above mixed-effects models, treatment and day were included as fixed effects, and, to control for repeated measures, focal pair was included as a random effect.

Amount of time females spent with both stimulus females over the hours of the preference test was compared using a LMM, with hour and treatment as fixed effects and female pair as a random effect. Additionally, we tested total time spent (seconds) with partner and stranger during the first hour with a LMM, again treatment and partner were fixed effects and female pair as a random factor. Here, we included a weight of total time with both females, to account for the difference across pairs. A *post hoc* test was used to assess within interaction significance using the multcomp package in R (Hothorn et al. 2008), with manually assigned contrasts. To assess latency to enter the first side and to start of the preference test, a LM was used, with treatment as the fixed effect. Time females spent in the middle cage and in total with a stimulus female was analysed with a LM, with treatment as a fixed effect.

Model assumptions were assessed for all models visually using diagnostic plots, and in the event that they were not fulfilled, data were transformed. Square root transformations were used in LMMs for positive time interacting during cohabitation, time spent with stimulus females in the partner preference test and latency to meet during the cohabitation phase, as well as in LMs for latency to enter first side of the preference set-up, and time spent in total with both stimulus females. All linear and mixed-effects models, unless otherwise stated, were conducted using the lme4 package in R (Bates et al. 2014). Furthermore, in all models, variance components were estimated using maximum likelihood ('ML') methods and additionally all random effects were kept in the models. We selected the minimal most adequate model through backward stepwise model selection, and significance of fixed terms was determined using likelihood ratio tests (Crawley 2007). Table 2 provides means ± standard error of the mean (SE) for time spent in the same cage and together as well as latencies for the cohabitation phase, and time spent in the middle cage and latency to start for the partner preference.

Table 2: Mean and standard error of the mean (SE) for analysis where no figure has been provided. Times given by treatment and day, except in the case of the partner preference where means are given only by treatment

	Cohabitation						Partner preference			
Beh	Same cage[a] (s)		Together[b] (s)		Latency to meet (s)		Latency to start (s)		Middle cage (s)	
Trt	OT	CON	OT	CON	OT	CON	OT	CON	OT	CON
Day										
1	4834.0 ± 391.4	4664.7 ± 404.5	3577.2 ± 393.7	3398.3 ± 397.1	362.0 ± 109.3	378.2 ± 72.8	972.3 ± 213.9	1171.7 ± 451.8	1888.0 ± 382.7	1849.6 ± 326.5
2	5631.6 ± 577.8	4393.3 ± 604.7	5112.9 ± 666.5	4053.4 ± 615.9	125.4 ± 38.4	75.3 ± 11.8				
3	5658.1 ± 724.1	5513.6 ± 546.8	5340.4 ± 769.2	5186.1 ± 509.2	136.7 ± 55.7	74.1 ± 21.2				

Trt, treatment; Beh, behaviour.
[a]Same cage = time females spent in the same cage as each other regardless of interaction type.
[b]Together = the time females spent together in the same cage minus any time interacting negatively.

Results

Phase A: Cohabitation

During the cohabitation phase, females did not differ significantly in the amount of time spent in the same cage across the 3 d (LMM: χ^2 (1) = 2.93, p = 0.231, Table 2), and there was no significant overall effect of treatment (LMM: χ^2 (1) = 1.02, p = 0.313). Of the time spent in the same cage, there was no significant effect of OT on socio-positive behaviours (LMM: χ^2 (1) = 0.53, p = 0.468); however, socio-positive behaviours increased significantly across the three treatment days in both groups (LMM: χ^2 (1) = 13.62, p = 0.001, Fig. 2). Furthermore, time spent in the same cage excluding negative behaviours (i.e. time spent interacting positively plus time in the same cage but not interacting, neutral behaviours) followed the same pattern, a non-significant effect of treatment (LMM: χ^2 (1) = 0.68, p = 0.411) and a significant increase across the 3 d (LMM: χ^2 (1) = 11.67, p = 0.003, Table 2).

Occurrence of socio-negative behaviours was generally rare, even during the first day, and did not differ significantly across the two treatment groups (GLMM: χ^2 (1) = 0.15, p = 0.701, Fig. 3), but there was a significant decline in the number of negative interactions across the 3 d (GLMM: χ^2 (1) = 54.17, p < 0.001). Treatment had no significant effect on the latency of the animals to meet (LMM: χ^2 (1) = 0.17, p = 0.679, latency to first interaction on the first and subsequent days, Table 2) but latency to meet decreased significantly across the 3 d of the experiment (LMM: χ^2 (1) = 28.77, p < 0.001). Furthermore, there was no significant effect of treatment on the proportion of time females spent in the same cage at the different time points measured throughout the night and day (GLMM: χ^2 (1) = 0.03, p = 0.870). Females were found in the same cage significantly more often than in separate cages across the 3 d (GLMM: χ^2 (1) = 7.85, p = 0.005).

Phase B: Partner Preference

Over the 9 h analysed for the preference test, the time females spent with the stimulus females differed significantly (LMM: χ^2 (8) = 16.02, p = 0.042), regardless of treatment (LMM: χ^2 (1) = 0.39, p = 0.531). Whereby, time spent with stimulus females decreased during the 2nd and 3rd hour tested but was otherwise similar in the remaining hours. Suggesting a 9-h observation period may not be necessary for determining preference in mice using this set-up. Given

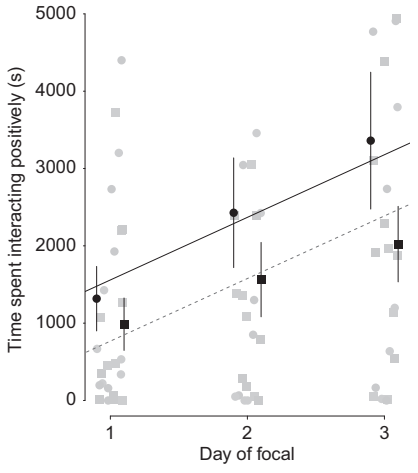

Fig. 2: Time spent interacting positively across the three treatment days. There was a significant increase in positive interactions across the three treatment days, in both treatments (p < 0.01). Dark symbols illustrate mean ± SE for positive interactions for each treatment per day; grey symbols indicate the total time per pair per day [solid line (circles): OT, dashed line (squares): CON].

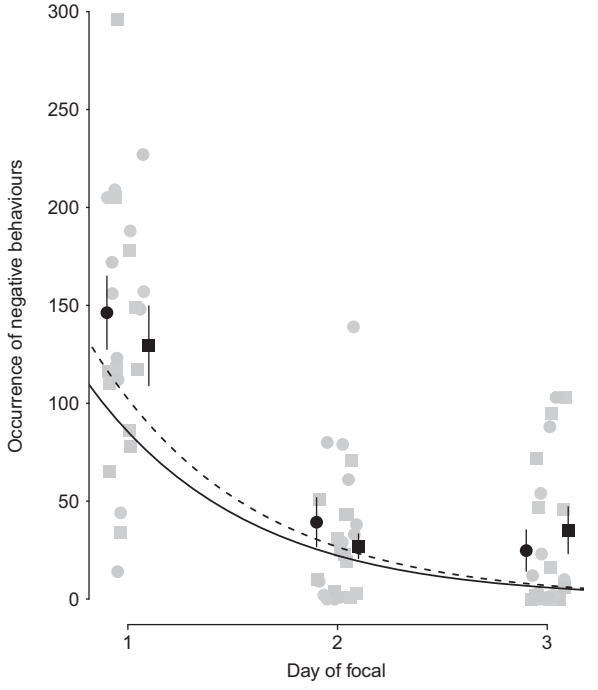

Fig. 3: Occurrence of negative behaviours across the three treatment days. There was a significant decrease in negative behaviours across the three treatment days, in both treatments (p < 0.01). Mean ± SE denoted by dark symbols for each treatment per day, grey symbols indicate the total occurrence per pair per treatment [solid line (circles): OT, dashed line (squares): CON].

this observation, we focused on the preferences displayed in the first hour, when time spent with females was above the overall average, and 18 of 22 females were in the same cage as their cohabitation partner for 50% or more of the time with a stimulus female (OT: 9/12, CON: 9/10).

During this first hour of the preference test, there was a significant interaction between treatment and partner, whereby CON females spent significantly more time with their partner than OT females (LMM: χ^2 (1) = 6.29, p = 0.012, Fig. 4). *Post hoc* tests revealed that OT females showed no significant difference in the amount of time spent with either stimulus female (LMM: $z = 1.49$, p = 0.327, Fig. 4). However, CON females spent significantly more time with their partner than the stranger (LMM: $z = 5.55$ p < 0.001, Fig. 4). Time spent in the middle cage during this hour did not differ significantly across the two treatments (LM: $F_{1,\ 20} = 0.01$, p = 0.941, Table 2).

Oxytocin and CON females did not differ significantly in the total time spent with another female regardless of whether she was the partner or stranger (LM: $F_{1,20} = 0.33$, p = 0.572). Treatment did not significantly affect the latency to enter the first side of the preference set-up (LM: $F_{1,20} = 1.42$, p = 0.248) or the latency to the start of the partner preference (LM: $F_{1,20} = 0.\ 18$, p = 0.678).

Discussion

Female house mice cooperatively raise communal litters and form partnerships that have been shown to increase reproductive success (König 1994). In the past, studies have suggested that time spent in close association can be used as a measure for social bonds among individuals (Silk et al. 2003; Weidt et al. 2008; Cameron et al. 2009; Schülke et al. 2010). Therefore, it could be assumed that female mice establish social bonds prior to communally nursing through spending time interacting positively with one another. Our study investigated whether injecting oxytocin (OT) could play a role in the facilitation of initial behaviours between unfamiliar females and influence female preference for a cohabitation partner. Contrary to our expectations, exogenous OT administration did not alter initial prosocial behaviours between two unfamiliar, unrelated and non-reproducing females and interfered in the formation of a preference for a familiar female.

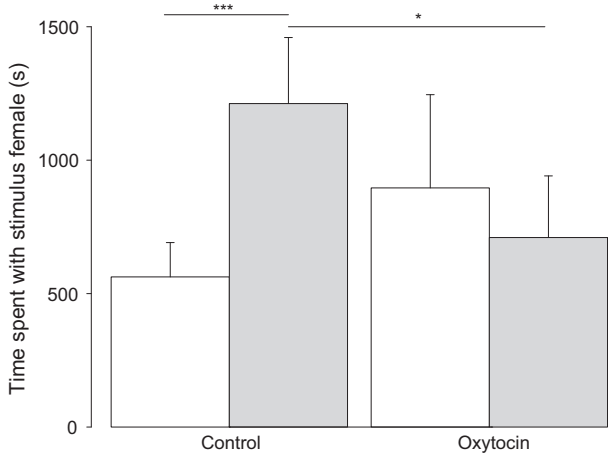

Fig. 4: Partner preference test. Time females spent (in the first hour), mean ± SE, in the cage with the partner (grey) and with the stranger (white) for each treatment, * < 0.05, *** < 0.001, ns = when no asterisks indicated.

Socio-positive behaviours significantly increased over the 3 d in both treatments, suggesting that females generally became more affiliative and tolerant of one another. However, in the subsequent preference test, it was only CON females who spent significantly more time in the same cage with the partner than the stranger, which implied they had formed a preference for their partner. Time spent close to or in the same cage as a conspecific is commonly interpreted as a preference and has been used in previous studies (Carter et al. 1992; Williams et al. 1994; Insel & Hulihan 1995; Young & Wang 2004). Despite OT females exhibiting the same pattern in their behaviours as CON females during the 3 d of cohabitation, they did not demonstrate a preference for their cohabitation partner when given a choice of partners. Instead, they spent similar amounts of time with both stimulus females, suggesting that OT interfered with the formation of a partner preference.

Treatment Effects on Female Behaviour

Oxytocin has been suggested to promote sociability and motivate individuals to affiliate more generally (Campbell 2008). This has been demonstrated in studies where animals treated with OT showed increased social contact (Witt et al. 1990; Carter et al. 1992), and increased exploratory behaviours (Dharmadhikari et al. 1997). While more attention has been given to the positive effects of OT, there are also negative ones (Beery 2015), and OT has been found to enhance both positive and negative perceptions (De Dreu et al. 2012; Crockford et al. 2014). Furthermore, the influence of OT has been related to interindividual competition (Radke & de Bruijn 2012), and when information related to a partner is inaccessible, OT

can reduce cooperation (Declerck et al. 2010). OT manipulation was demonstrated to prevent stress-induced social avoidance in rats and facilitated a social preference for novel conspecifics in both rats and mice (Lukas et al. 2011). However, Peñagarikano et al. (2015) found that during a social interaction test, pairs of wild-type mice treated with OT did not differ in time spent interacting socially on first encounter with a stranger, when compared against control-treated pairs. These findings support those of the current study, as OT did not influence time spent with a stranger on first encounter or facilitate a preference for the previous partner. Additionally, in female meadow voles, OT increased time spent huddling with a preferred partner but was not required for social preference formation, as control females formed a preference regardless of treatment (Beery & Zucker 2010). Results from our study support such findings, as OT did not increase social interactions between pairs of new, unfamiliar individuals, beyond those that would be expected naturally. Taken together, these results, combined with findings from the current study, support the idea that elevated endogenous OT is not a facilitator in initial social interactions among female house mice. Consequently, OT does not seem to affect the process of females becoming familiar, yet when compared against the CON, it hinders the formation of a preference.

Some alternative explanations for the OT females in the current study spending similar amounts of time with both stimulus females could be that they were showing increased exploratory behaviour (Dharmadhikari et al. 1997; Windle et al. 1997; Lukas et al. 2011). Windle et al. (1997) found that mildly stressed rats treated with OT spent a higher proportion of time in the open arms of an elevated maze. Furthermore, Uvnäs-Moberg et al. (1994) found high doses of peripherally administered OT to increase the amount of time individuals spent away from the perceived security of a boundary wall. Additionally, OT has been suggested to have anxiolytic effects on behavioural systems, which could moderate the anxiety response to stress (Windle et al. 1997; Smith & Wang 2014), and injection with OT was demonstrated to have antistress effects comparable to those produced by positive social stimuli (Uvnäs-Moberg 1998). This could suggest that the focal female considered the unfamiliar female less aversive and easier to approach during the preference test. However, in the current study, we did not explicitly test for exploratory behaviour or a stress response; therefore, future research could investigate such hypotheses. OT has also been demonstrated to be an essential peptide for facilitating familiarity recognition and the ability to distinguish familiar from unfamiliar conspecifics (Choleris et al. 2003, 2009). OT in our study may have facilitated discrimination of familiar from unfamiliar individuals and could even have enhanced the focal female's response to the unfamiliar partner.

Past studies have shown that some behavioural effects of OT could be linked to the activation of vasopressin receptors (reviewed in: Freeman & Young 2016), such as the V1a receptor (Busnelli et al. 2013; Bowen & McGregor 2014). In the current study, this could suggest that OT interfered with preference formation through binding at the V1a receptors. Given that studies using similar doses and routes of administration have lead to contrary findings (Cushing & Carter 2000; Bowen & McGregor 2014), we cannot discard the possibility that OT administration in our study also acted through vasopressin receptors.

The Importance of Social Preferences

It has been shown that female house mice preferentially allonurse with kin or familiar females and in doing so maximise their reproductive success (König 1994). However, females also develop non-random preferences for social partners when kept in groups of unrelated females, with roughly three-quarters of females showing significant associations with at least one other female (Weidt et al. 2008). Furthermore, Weidt et al. (2008) demonstrated that female house mice have greater reproductive success with preferred over un-preferred partners when both are unrelated. Despite females, in the current experiment, not getting the opportunity to choose their initial partner, we found a steady increase in positive interactions across the 3 d, suggesting females became more affiliated. Additionally, OT females behaved similarly to CON females during cohabitation, but when presented with a novel, unknown female, they became choosier, supporting the idea that perhaps OT increased social approach behaviour (Lim & Young 2006) or increased salience of social stimuli (Young & Barrett 2015). Further research would be required to disentangle such ideas as well as determine whether females would go on to form a communal nest together, and whether OT can play a role in this.

Route of Administration

When interpreting our results, it must be considered that the peripheral administration of OT may not have crossed the blood–brain barrier. Findings from previous studies, however, suggest small quantities may do

so. Dosage of OT for this study was derived from previous research, in particular the findings of Neumann et al. (2013) who found increased OT in brain dialysates 30 min post-intraperitoneal injection in mice. Their findings provided initial evidence for the uptake of peripherally administered synthetic OT into the brain, although the routes of entry were unknown (Neumann et al. 2013). These results are supported by additional studies that used peripheral administration of OT to assess its behavioural effects. Mooney et al. (2014) found increased huddling behaviour and time in close proximity to conspecifics in the naked mole rat. Meerkats injected subcutaneously with OT demonstrated increased cooperative behaviours such as digging and pup feeding as well as decreased initiation of aggression (Madden & Clutton-Brock 2011), and peripheral OT administration inhibited infanticide in female house mice (McCarthy 1990). Additionally, intraperitoneal injection of OT significantly increased time in social contact with a novel partner in Cntnap2 mutant mice compared against the vehicle control (Peñagarikano et al. 2015). Cntnap2 mice have social behaviour deficits linked to autism and reduced expression of OT within their neurons; therefore, this increase in social behaviour suggests OT may have crossed the blood–brain barrier (Peñagarikano et al. 2015). Therefore, these past studies among others suggest peripheral administration of OT to be justifiable as a method of manipulation and to assess its effects on social behaviours.

Conclusions

Our results demonstrate that unfamiliar female house mice naturally became more affiliative towards one another over time (as demonstrated by the CON) and that this process appears not to be affected by treatment with OT. However, our results also suggest that injecting exogenous OT prevented the formation of a preference for a cohabitation partner. Additional research should be carried out to investigate this further, by measurement of peripheral and central OT levels, or testing whether behaviours are reversed when an OT antagonist is introduced. These results contribute to our growing knowledge of OT and its variable influence on social behaviour; they support findings that suggest its effects can be very context and perhaps species specific (Insel & Young 2001; Campbell 2008; Donaldson & Young 2008; Radke & de Bruijn 2012). Additionally, they support the idea that the role of OT can be influenced by many factors including other hormonal effects (discussed in: Campbell 2008) and contact with a known or preferred partner (Beery & Zucker 2010; Crockford et al. 2013). Lim & Young (2006) discuss how attachment bonds can be both 'selective and enduring' between individuals and social bonds require a combination of many processes. Consequently, with regard to social partner preferences among female house mice, there remains plenty of scope to discover more about their social behaviour and factors that may influence choice of social partner.

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